APPENDIX III

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SCATTER FACTOR AND ANGIOGENESIS

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I. Introduction: Scatter Factor (Hepatocyte Growth Factor) and the c-*met* Receptor

A. Scatter Factor (SF)

SF is a mesenchyme-derived protein that dissociates ("scatters") sheets of epithelium (Stoker *et al.*, 1987; Rosen *et al.*, 1989). SF is identical to hepatocyte growth factor (HGF) (Weidner *et al.*, 1991a; Bhargava *et al.*, 1992), a serum mitogen for rat hepatocytes that is thought to function as an hepatotrophic factor for liver repair (Miyazawa *et al.*, 1989; Nakamura *et al.*, 1989). SF is a heparin-binding glycoprotein consisting of a 60-kDa α-chain and a 30-kDa β-chain (Gherardi *et al.*, 1989; Rosen *et al.*, 1990b; Weidner *et al.*, 1990). The α-chain contains an N-terminal hairpin loop and four "kringles" (disulfide looped structures that mediate protein–protein interactions). The β-chain is homologous to serine proteases. SF has 38% amino acid sequence identity to the proenzyme plas-

minogen (Nakamura *et al.*, 1989), but lacks protease activity (Rosen *et al.*, 1990b) due to the replacement of two essential amino acids at the catalytic triad of the β-chain. SF is synthesized as a 728-amino-acid precursor (preproSF); intracellular cleavage of a 31-amino-acid signal peptide results in its secreted single-chain form (proSF), which is biologically inactive (Lokker *et al.*, 1992). Extracellular cleavage of proSF at ^{494}Arg–^{495}Val yields active two-chain SF. HGF activator, a novel serine protease homologous to coagulation factor XII (Hagemann factor), may be a physiologic cleavage enzyme for SF (Miyazawa *et al.*, 1993). This enzyme is produced in zymogen form; it may be activated by a proteolytic cascade initiated by tissue injury (Miyazawa *et al.*, 1994). Plasminogen activators (uPA and tPA) can also cleave and activate proSF, but only at supraphysiological concentrations (Naldini *et al.*, 1992; Mars *et al.*, 1993).

B. c-*met* Receptor

The SF receptor is the protein product of the *c-met* protooncogene (Bottaro *et al.*, 1991), a transmembrane tyrosine kinase (TK) expressed predominantly by epithelia (Gonzatti-Haces *et al.*, 1988). The *c-met* receptor is a 190-kDa glycoprotein consisting of a 145-kDa membrane-spanning β-chain and a 50-kDa α-chain that is expressed on the cell surfac The extracellular binding, transmembrane, and intracellular kinase, and noncatalytic phosphate acceptor domains are located on the β-chain. Studies suggest that much of the signal transduction from the SF-activated *c-met* receptor occurs through the interaction of a novel tandem YV(H/N)V motif with the *src* homology-2 (SH2) domains of various intracellular signaling molecules (Ponzetto *et al.*, 1994). Tyrosine phosphorylation at this site mediates the binding of *c-met* to phosphatidylinositol-3′-kinase, protein tyrosine phosphatase 2, phospholipase Cγ, pp60$^{c\text{-}src}$, and the *grb2/hSos1* complex. Two receptor TKs related to *c-met*, *c-sea*, and *Ron* have been described (Ronsin *et al.*, 1993; Huff *et al.*, 1994). The ligand for the *Ron* receptor was identified as a macrophage-stimulating protein (Wang *et al.*, 1994), a kringle protein with 50% sequence identity to SF (Yoshimura *et al.*, 1993). The ligand for *c-sea* has not yet been described.

C. Biologic Activities of SF

SF transduces three major classes of cellular actions *in vitro*: motility, growth, and morphogenesis. Studies employing chimeric receptor constructs indicate that each of these actions can be transduced by the *c-met*

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II. SF Biologic Acti
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A. Vascular Endothelial

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TK (Weidner *et al.*, 1993; Zhu *et al.*, 1994). In addition to cell dissociation, SF induces random movement of isolated epithelial cells, chemotactic (gradient-directed) migration, migration from carrier beads to flat surfaces, and invasion through extracellular matrix proteins (Rosen *et al.*, 1990b,c, 1991a; Weidner *et al.*, 1990; Bhargava *et al.*, 1992; Li *et al.*, 1994). SF stimulates the mRNA and protein expression of both uPA and uPA receptor (uPAR) (Pepper *et al.*, 1992; Grant *et al.*, 1993; Rosen *et al.*, 1994b). The net effect is to increase the amount of uPA bound to uPAR on the cell surface. Receptor-bound uPA on the cell surface is catalytically active and is thought to mediate focal degradation of the extracellular matrix necessary to clear a path for invading cells (Saksela and Rifkin, 1988). Thus, SF appears to be able to "switch on" a program of cell activities for invasion. The switching mechanism may involve induction of an as yet unidentified transcription factor. SF is mitogenic for various normal cell types, including epithelial cells, vascular endothelial cells, and melanocytes (Kan *et al.*, 1991; Rubin *et al.*, 1991; Halaban *et al.*, 1992). SF is also a potent morphogen. SF induces MDCK epithelial cells incubated in collagen I gels to organize into a network of branching tubules with the proper apical–basolateral polarity (Montesano *et al.*, 1991; Santos *et al.*, 1993). Similarly, SF induces mammary epithelial cells to form ductlike structures (Tsarfaty *et al.*, 1992). Thus, SF can activate specific programs of cell differentiation depending upon the cell type and environment.

II. SF Biologic Actions on Blood Vessel Wall Cells *in Vitro* and *in Vivo*

A. Vascular Endothelial Cells (ECs)

1. EC as a SF Producer Cell

Vascular ECs may function both as a producer of SF and as a target of SF action. ECs *in vitro* and *in vivo* normally produce little or no SF (Stoker *et al.*, 1987; Rosen *et al.*, 1989; Matsumoto *et al.*, 1993). However, following liver injury, hepatic sinusoidal ECs as well as pulmonary alveolar ECs synthesize SF, as demonstrated by *in situ* hybridization to detect SF-specific mRNA transcripts (Yanagita *et al.*, 1992; Noji *et al.*, 1990). These findings suggest that appropriately stimulated ECs are capable of producing SF. They further suggest that paracrine and endocrine signals associated with tissue injury are responsible for the induction of EC synthesis of SF. Some of the putative factors that may transmit such a signal are discussed later (Section III.C.3).

2. EC as an SF Target Cell

During the early stages of angiogenesis *in vivo*, ECs from preexisting small vessels (usually venules that lack a smooth muscle covering) focally degrade the subendothelial basement membrane, migrate out into the interstitium toward an angiogenic stimulus, and form capillary sprouts (Folkman, 1985). Sprouting ECs proximal to the migrating tip proliferate; subsequently, the EC sprouts organize into an anastamosing network of capillary tubes. Finally, these ECs synthesize new basement membrane. Adhesion of SMCs and pericytes and formation of new basement membrane are processes associated with the termination of the angiogenic response (Folkman, 1985; Antonelli-Orlidge *et al.*, 1989). Stimulation of EC motility, proliferation, and capillary-like tube formation *in vitro* are thought to correlate with the ability to induce angiogenesis *in vivo*, since each of these processes occurs during the formation of new blood vessels (Folkman, 1985).

Both large vessel- and microvessel-derived ECs express the c-*met* receptor and are biologically responsive to SF (Rosen *et al.*, 1990b,c, 1991b; Bussolino *et al.*, 1992; Grant *et al.*, 1993; Naidu *et al.*, 1994). SF is chemotactic to ECs and stimulates random motility, as demonstrated in assays using microwell modified Boyden chambers (Rosen *et al.*, 1990b, 1991b). In addition, SF induces the migration of ECs cultured on microcarrier beads from the beads to flat culture surfaces (Rosen *et al.*, 1990b,c). In chemoinvasion assays, a gradient of SF induces the penetration of ECs through porous filters coated with Matrigel, a reconstituted matrix of basement membrane (Rosen *et al.*, 1991b). Maximal chemotaxis, bead migration, and invasion of human umbilical vein ECs (HUVEC), calf pulmonary artery ECs (CPAE), bovine aortic ECs (BAEC), and bovine brain ECs (BBEC) are typically observed at SF concentrations of 2–20 ng/ml. In studies using the microcarrier bead migration assay, we found that BBEC migration was stimulated 5-fold by SF, but was unaffected by basic FGF or EGF (Rosen *et al.*, 1991c). On the other hand, TGFβ blocked both basal and SF-stimulated migration of BBEC. Migration from carrier beads was blocked by inhibitors of protein synthesis (cycloheximide), but not by inhibitors of DNA synthesis (hydroxyurea) (Rosen *et al.*, 1991c).

In addition to motility, SF stimulates DNA synthesis and proliferation of some EC types, including HUVEC and human omental microvessel ECs (Rubin *et al.*, 1991; Morimoto *et al.*, 1991). Capillary tube formation appears to be an independent property of ECs not directly related to motility or proliferation (Grant *et al.*, 1989). When ECs are plated onto a surface of reconstituted basement membrane (Matrigel), they cease DNA synthesis and proliferat and begin to organize into a r lates capillary-like tube format to 5–10-fold, as determined b stained cultures (Rosen *et al.*,

SF also induces large increas cultures (Rosen *et al.*, 1991b; C uPA activity is cell-associated r associated uPA is bound to uP well-positioned to mediate foca teins, a prerequisite for invasi gether, these findings indicat phenotypic characteristics exp lustrated in Fig. 1).

B. Vascular Smooth Muscl and Pericytes

1. SMC

In vitro, bovine aortic, hum duce SF at rates comparable fibroblasts (e.g., MRC5, WI3 (Rosen *et al.*, 1989, 1990a). T SMC-derived SF are very simil is likely that these molecules

2. SMC

Psoriasis is a chronic inflan proliferation of pidermal ke mal papillae and papillary d cytes, ECs) in psoriatic plaque *al.*, 1993), suggesting that th SF. Smooth muscle cells in t levels of immunoreactive SF pericytes (which are generall essential component of angio newly formed vessels, thus co process (Antonelli-Orlidge *et* SF itself might induce the infl appropriate time during ang

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DNA synthesis and proliferation, extend long cytoplasmic processes, and begin to organize into a network of capillary-like tubes. SF stimulates capillary-like tube formation in HUVEC and BBEC cultures by up to 5–10-fold, as determined by computerized digital image analysis of stained cultures (Rosen *et al.*, 1991b; Grant *et al.*, 1993).

SF also induces large increases in the expression of uPA activity by EC cultures (Rosen *et al.*, 1991b; Grant *et al.*, 1993). Most of the SF-induced uPA activity is cell-associated rather than secreted. The majority of cell-associated uPA is bound to uPA receptor on the cell surface, where it is well-positioned to mediate focal degradation of extracellular matrix proteins, a prerequisite for invasion (Saksela and Rifkin, 1988). Taken together, these findings indicate that SF can induce most or all of the phenotypic characteristics expected of ECs undergoing angiogenesis (illustrated in Fig. 1).

B. Vascular Smooth Muscle Cells (SMCs) and Pericytes

1. SMC as a SF Producer Cell

In vitro, bovine aortic, human iliac artery, and rat arterial SMCs produce SF at rates comparable to those of high producer human lung fibroblasts (e.g., MRC5, WI38) (64–128 scatter units/10^6 cells/48 hr) (Rosen *et al.*, 1989, 1990a). The biological and chemical properties of SMC-derived SF are very similar to those of fibroblast-derived SF, and it is likely that these molecules are identical (Rosen *et al.*, 1990a,b).

2. SMC as an SF Target Cell

Psoriasis is a chronic inflammatory skin disease characterized by the proliferation of epidermal keratinocytes and neovascularization in dermal papillae and papillary dermis. Cells of the microvessel wall (pericytes, ECs) in psoriatic plaques stain positively for c-*met* protein (Grant *et al.*, 1993), suggesting that these cell types are potential target cells for SF. Smooth muscle cells in tumor microvasculature also express high levels of immunoreactive SF (Section IV.B). Recruitment of SMCs and pericytes (which are generally regarded as microvascular SMCs) is an essential component of angiogenesis. These cells are thought to stabilize newly formed vessels, thus contributing to termination of the angiogenic process (Antonelli-Orlidge *et al.*, 1989). Therefore, it seems logical that SF itself might induce the influx and/or proliferation of these cells at the appropriate time during angiogenic response.

Cultured pericytes from bovine retina express c-*met* mRNA, as de-

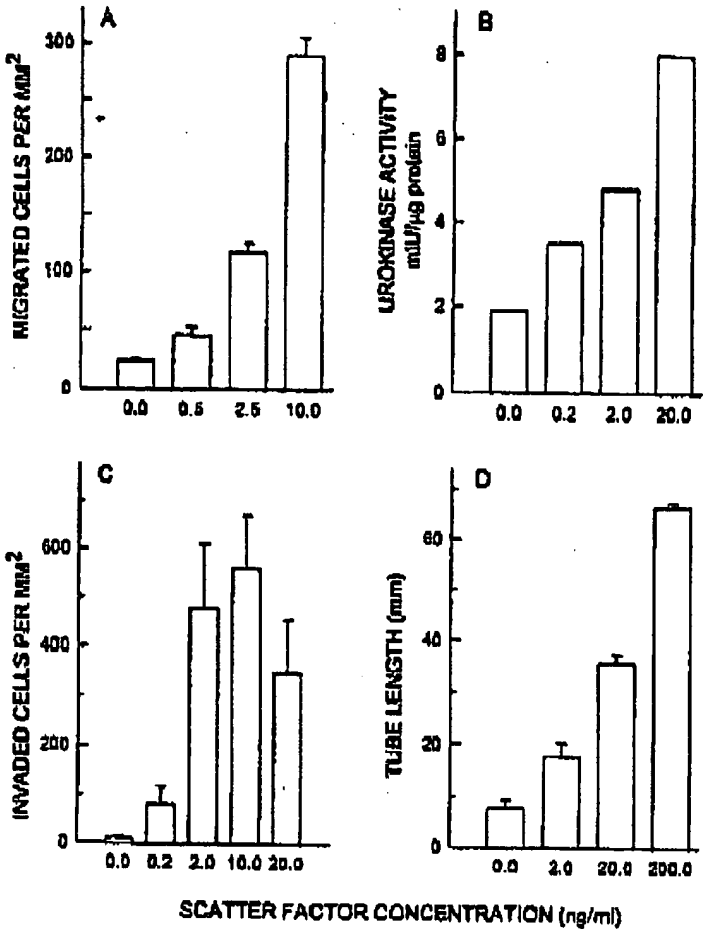

FIG. 1. Angiogenic endothelial cell (EC) phenotype induced by SF. EC chemotaxis, protease production, invasion, and capillary-like tube formation, processes required during angiogenesis, are each stimulated by SF. (A) Chemotaxis. CPAEs were induced to migrate across collagen-coated 8-μm Nucleopore filters by SF in the lower wells of microwell modified Boyden chambers. Assays were performed as described by Rosen *et al.* (1990b). Values plotted represent the numbers of cells migrating from the upper wells to the underside of the filter during a 6-hr incubation (mean ± SEM of triplicate assays). (B) Urokinase production. Confluent BBECs were incubated with SF for 24 hr. The medium was replaced with fresh DMEM (0.1ml/cm²), and the cells were incubated for 8 hr. The 6-hr-conditioned medium was collected, and the cells were lysed. Plasminogen activator activity was assayed as described by Grant *et al.* (1993), and the total activity (secreted plus cell-associated) was normalized per microgram of cell protein. Nearly all of this activity was blocked by antiurokinase antibodies, indicating that it is due to uPA rather than tPA. (C) Invasion. BBECs were induced to migrate invasion through porous filters coated with a basement membrane matrix (Matrige chambers. Assays were performed a represent the numbers of cells migra incubation (mean ± SEM of triplicat were induced to form tubes on Matri described by Rosen *et al.* (1991b). Val hr incubation, as determined by com assays).

termined by reverse transcrip Rosen and M. Park, unpublis finding that pericytes expres Moreover, we have found that aortic SMCs and bovine retina degrees of stimulation of pro calf serum were 2.4- and 1.8-f cytes, and 1.7- and 1.5-fold fo maximal values were observed consistent with the putative re ence of SMCs in new microve

The ability of SMCs to pro may function as an autocrine the phenotype of cultured S SMCs present in normal adult *vivo* are usually quiescent, but lar injury. A parallel phenome when SMCs are explanted fro and Chamley-Campbell, 1981 by transition from a quiesc synthetic-proliferative phenot teristic of proliferating injure tractile SMCs. It seems unlik duce significant amounts of SI mediate paracrine (SMC–EC) associated with the vascular re

C. *In Vivo* Angiogenic Acti

We used two different assa cornea assay, to demonstrate t formation *in vivo* (Grant *et al.*

termined by reverse transcriptase PCR analysis of pericyte RNA (E. M. Rosen and M. Park, unpublished results), which is consistent with the finding that pericytes express immunoreactive c-*met* protein *in vivo*. Moreover, we have found that SF stimulates the proliferation of bovine aortic SMCs and bovine retinal pericytes *in vitro* (Fig. 2). The maximum degrees of stimulation of proliferation in media containing 1 and 5% calf serum were 2.4- and 1.8-fold for SMCs, 1.7- and 2.2-fold for pericytes, and 1.7- and 1.5-fold for bovine capillary ECs, respectively. These maximal values were observed at 20–100 ng/ml SF. These findings are consistent with the putative role of SMCs in angiogenesis and the presence of SMCs in new microvessels induced by SF (Section II.C).

The ability of SMCs to produce and respond to SF suggests that SF may function as an autocrine growth factor for this cell type. However, the phenotype of cultured SMCs does not necessarily reflect that of SMCs present in normal adult large blood vessels. Normal adult SMCs *in vivo* are usually quiescent, but can be stimulated to proliferate by vascular injury. A parallel phenomenon called phenotypic modulation occurs when SMCs are explanted from vessels and passages *in vitro* (Campbell and Chamley-Campbell, 1981). Phenotypic modulation is characterized by transition from a quiescent contractile phenotype to an active synthetic-proliferative phenotype. Thus, cultured SMCs may be characteristic of proliferating injured SMCs rather than nonproliferating contractile SMCs. It seems unlikely that normal, unstimulated SMCs produce significant amounts of SF *in vivo*. Our findings suggest that SF may mediate paracrine (SMC–EC) and autocrine (SMC–SMC) interactions associated with the vascular response to injury.

C. *In Vivo* Angiogenic Activity

We used two different assays, the mouse Matrigel assay and the rat cornea assay, to demonstrate the ability of SF to induce new blood vessel formation *in vivo* (Grant *et al.*, 1993; Naldu *et al.*, 1994). In the former,

basement membrane matrix (Matrigel) by SF in the lower wells of 0.2-ml blind well Boyden chambers. Assays were performed as described by Bhargava *et al.* (1992). Values plotted represent the numbers of cells migrating to the lower surfaces of the filters during a 48-hr incubation (mean ± SEM of triplicate assays). (D) Capillary-like tube formation. HUVECs were induced to form tubes on Matrigel-coated surfaces by SF. Assays were performed as described by Rosen *et al.* (1991b). Values plotted represent the total tube length after a 24-hr incubation, as determined by computerized digital imaging (mean ± range of duplicate assays).

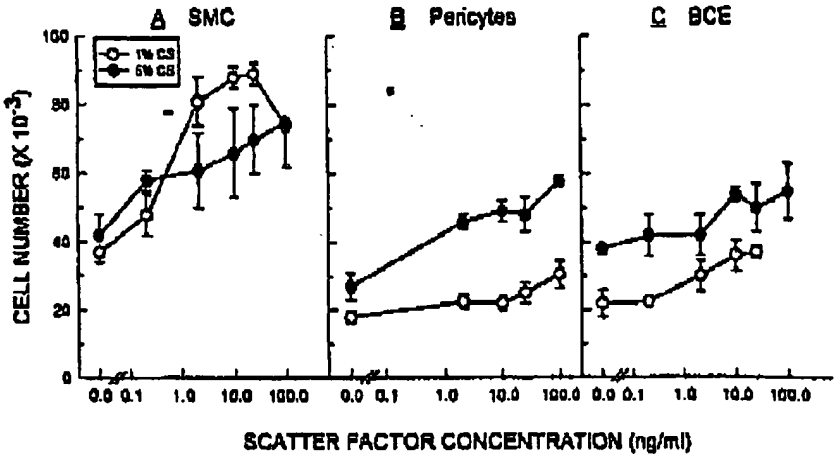

FIG. 2. Effect of SF on proliferation of vascular cell types. Bovine aortic smooth muscle cells (SMCs) (A), bovine retinal pericytes (B), or bovine adrenal capillary endothelial cells (BCEs) (C) were seeded into 2-cm² culture wells at 1×10^4 (SMCs, BCEs) or 2×10^4 (pericytes) cells per well in DMEM containing 10% calf serum. The cells were allowed to attach overnight, after which the medium was replaced with DMEM containing 1 or 5% calf serum and the indicated dose of SF. Cells were incubated for 3 (SMCs, BCEs) or 6 days (with one refeeding on day 3) for slower growing pericytes. Values represent cell counts from triplicate wells (mean ± SD).

different doses of SF were mixed with 0.5 ml of Matrigel in the liquid state at 4°C. The Matrigel was injected subcutaneously into either XID nude beige mice or C57/BL mice. At body temperature, Matrigel rapidly forms a solid gel, retaining the SF and allowing prolonged exposure of the surrounding tissues to it. Animals were sacrificed after 10 days, and the ingrowth of blood vessels into the Matrigel plugs was quantitated by computerized digital image analysis of histological sections stained with Masson's trichrome. Angiogenesis assessed at day 10 increased in a dose-dependent manner from 2–200 ng/ml SF to 4–5 times control values. Responses were quantitatively similar in nude mice and C57/BL mice. Inflammatory responses were not observed in nude mice at any SF dose and were found only at supramaximal SF doses (≥2000 ng/ml) in C57/BL mice.

In the second assay, SF was dissolved in Hydron polymer, and dried Hydron pellets were placed in surgically created pockets about 1.5 mm from the limbus of the avascular rat cornea. Animals were perfused with colloidal carbon and sacrificed after 7 days. The growth of new vessels from the limbus toward the pellet was assessed. In these assays, SF in-

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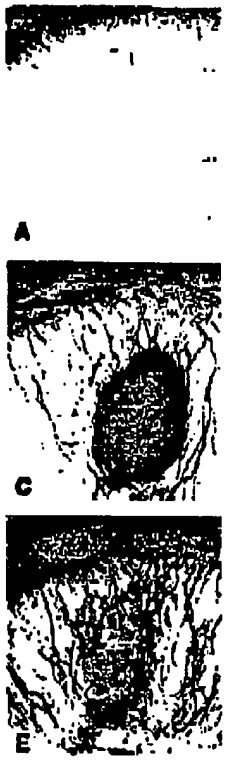

FIG. 3. Neovascular responses indu
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from the limbus. After 7 days, corne
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the implant. No angiogenic responses
while at 50 ng of SF, the response was
seen at 100 and 500 ng of SF (C and D
basic FGF (E), a positive control. These
of capillary sprouts and hairpin loop
factor, 50 ng; C, Scatter factor, 100 n

duced dose-dependent corneal neovascularization in a fashion similar t that observed in the murine assay (Fig. 3). Purified native mouse SF and recombinant human SF induced equal angiogeneic responses, and the maximal responses induced by SF were similar in intensity to that induced by basic FGF (Grant *et al.*, 1993). Antibodies against SF blocked SF-induced angiogenesis but did not affect FGF-induced angiogenesis.

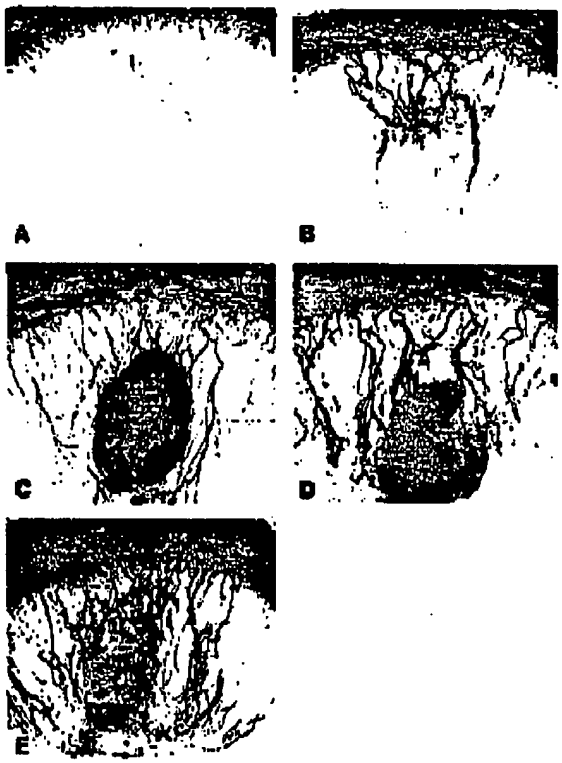

FIG. 9. Neovascular responses induced in the rat cornea by purified mouse SF. Hydron pellets containing test samples were implanted into rat corneas to allow ingrowth of vessels from the limbus. After 7 days, corneas were perfused with colloidal carbon, and whole mount preparations were photographed. The darkened central area is the reflection of the implant. No angiogenic responses were observed in control pellets containing PBS (A), while at 50 ng of SF, the response was weak but positive (B). Strong positive responses were seen at 100 and 500 ng of SF (C and D), which are comparable to that induced by 150 ng of basic FGF (E), a positive control. These responses consist of sustained directional ingrowth of capillary sprouts and hairpin loops surrounding the implants. A, Control; B, Scatter factor, 50 ng; C, Scatter factor, 100 ng; D, Scatter factor, 500 ng; E, Basic FGF, 150 ng.

Inflammatory responses, assessed by F4/80 immunostaining to detect monocyte–macrophage infiltration, were observed only at supramaximal doses of SF. Therefore, SF appears to be at least as potent an inducer of angiogenesis as basic FGF.

Our findings indicate that the recruitment of inflammatory cells does not play a major role in SF-induced angiogenesis. However, in the mouse Matrigel assay, histologic sections prepared at early times (days 2–3) revealed many SMC/pericyte-like cells present in the Matrigel. Moreover, at higher doses of SF, histologic sections prepared on day 10 revealed SMCs in some of the newly formed vessels in the Matrigel (Grant *et al.*, 1993). Thus, SF-induced angiogenesis appears to be mediated by direct effects on ECs and, in addition, by direct and/or indirect effects on SMCs.

III. SF as a Potential Tumor Angiogenesis Factor

A. Angiogenesis in Human Cancers

Recent clinical studies suggest that tumor angiogenesis, as indicated by increased numbers of microvessels in the tumor stroma, is a strong independent indicator of poor prognosis in patients with invasive breast cancer (Weidner *et al.*, 1991b, 1992; Bosari *et al.*, 1992; Toi *et al.*, 1993). A subset of patients with noninvasive breast tumors [ductal carcinoma *in situ* (DCIS)] also exhibit elevated microvessel counts. Increased vessel count in DCIS patients is highly associated with other features suggestive of aggressive tumor biology (e.g., comedo subtype of DCIS, HER2/neu oncoprotein expression, and high Ki81 proliferation index) (Guidi *et al.*, 1994). Experimental studies of human and animal tissues indicate that an angiogenic phenotype may be observed in even earlier lesions (e.g., hyperplasia or dysplasia) of breast or other tissues (Brem *et al.*, 1978; Folkman *et al.*, 1989). It has not been established whether angiogenesis is required for early progression from the noninvasive to invasive cancer phenotype or whether it merely reflects an underlying aggressive tumor biology. However, various studies suggest that angiogenesis is a critical requirement for local growth and metastasis of established solid tumors (Folkman, 1992).

Whereas physiological angiogenesis in normal adult tissues (e.g., as occurs during wound healing, corpus luteum formation, and placental implantation) is tightly regulated spatially and temporally, tumor angiogenesis is characterized by persistent, abnormal neovascularization. A modest number of growth factors and cytokines are capable of inducing

angiogenesis in various *in vivo*
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B. Expression of SF within

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angiogenesis in various *in vivo* and *in vitro* assay systems [e.g., angiogenin, FGFs, EGF/TGFα, IL-8, PDECGF (platelet-derived endothelial cell growth factor), SF, TNFα, TGFβ, and VEGF]. These angiogenic factors may be produced by tumor cells, host stromal cells (e.g., fibroblasts and SMCs), or infiltrating leukocytes (e.g., lymphocytes, macrophages, and mast cells) (Polverini, 1989; Leek *et al.*, 1994). The precise mechanisms leading to angiogenesis in human cancers, the specific angiogenic factor(s) involved, and the cell types that produce them are not well-delineated.

B. Expression of SF within Tumors

1. Carcinomas

Both SF and c-*met* appear to be up-regulated and down-regulated in precisely coordinated patterns during normal developmental and reparative processes (Sonnenberg *et al.*, 1993; Matsumoto and Nakamura, 1993; Joannidis *et al.*, 1994). On the other hand, SF is chronically overexpressed in tumors (Rosen *et al.*, 1994b; Yamashita *et al.*, 1994; Joseph *et al.*, 1995). As described in the following, overproduction of SF within tumors may be due, in part, to the accumulation of specific SF-inducing proteins. A high titer of SF in extracts of primary invasive breast carcinomas was found to be a powerful *independent* predictor of relapse and death (Yamashita *et al.*, 1994). In patients with transitional cell bladder cancers, higher titers of SF were found in high grade, muscle-invasive cancers than in low grade noninvasive or superficially invasive cancers (Joseph *et al.*, 1995). Patients in the former category usually fared poorly in comparison with patients in the latter category.

Since both SF content and tumor angiogenesis are strong independent prognostic indicators for breast carcinoma, it is reasonable to speculate that SF may be functioning as a breast cancer angiogen. If, indeed, SF functions as a tumor angiogen, then future studies should reveal a close correlation between tumoral SF content and the quantitative extent of tumor angiogenesis. Such a correlation may not be exact since, as described earlier, various other factors may contribute to tumor angiogenesis. Moreover, several naturally occurring protein factors, including thrombospondin (TSP1) and platelet factor-4, function as inhibitors of angiogenesis (see the following). It is also likely that SF may interact additively or synergistically with other angiogenesis-inducing factors, such as VEGF. Since many or most of these factors are found in tumors, the net angiogenic phenotype of the tumor may be determined by the balance of proangiogenic and antiangiogenic factors present.

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HGF-HGFR/c-*met* SYSTEM AND CARCINOGENESIS

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Many growth factors have been shown to transform cells by autocrine or paracrine mechanisms. With hepatocyte growth factor (HGF), however, all attempts to demonstrate the transforming activity have been unsuccessful using fibroblast systems. However, in a uniquely immortalized but not fully transformed mouse hepatocyte line MLE-10, we succeeded in demonstrating the transforming activity of HGF.

Full-length rat HGF cDNA under the control of a cytomegalovirus promoter was transfected into mouse liver epithelial-10 (MLE-10) cells and 5 HGF-producing lines were obtained. All 5 lines became growth capable in soft agar and 2 lines expressing HGF at relatively high levels produced tumors in the nude mouse subcutis. Since soft agar colony formation was blocked by anti-HGF antibody and MLE-10 cells express the HGF receptor at a high level, this transformation was concluded to be achieved through an autocrine mechanism. HGFR/c-*met* is expressed relatively frequently in malignant tumors, and serum or tissue HGF levels are elevated under various pathological conditions. The HGF-HGFR/c-*met* system, therefore, may be making an appreciable contribution to carcinogenesis in some specific cases.

Growth factors and their receptors play an important role in carcinogenesis. Thus, transforming growth factors α, β (TGFα, β), epidermal growth factor (EGF), and insulin-like growth factor (IGF) have all been shown to affect the genesis of gastric, colonic, esophageal and head and neck cancers by auto- or paracrine mechanisms (*7*, *32*, *34*). Hepatocyte growth factor (HGF) was initially identified as a substance stimulating growth of hepatocytes *in vitro* (*6*, *23*, *42*) and, after cloning of the cDNA (*20*, *21*), the c-*met* proto-oncogene was identified as its receptor (HGFR) (*1*, *26*). Subsequently, however, many other actions of HGF have become apparent (*18*, *25*; Matsumoto and Nakamura, in this volume): this factor stimulates the growth not only of hepatocytes but also of other types of cultured cells including melanocytes and renal tubular cells (*9*, *10*, *17*, *29*), is identical with the scatter factor (*4*, *5*, *13*, *33*), acts as a morphogen for kidney epithelial cells (*21*, *22*), and inhibits the growth of certain carcinoma and sarcoma cell lines *in vitro* and *in vitro* (*8*, *31*, *36*).

HGF is expressed, together with/without the HGF receptor HGFR/c-*met*, in some types of human cancers (*30*, *31*, *41*). Serum HGF levels are elevated in patients suffering from chronic hepatitis or liver cirrhosis (*39*) and in animals being treated with a hepatopromoter (*16*). The HGF receptor is also physiologically expressed in many types of cells, especially those of an epithelial nature (*37*), and is, in addition,

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frequently overexpressed or amplified in many types of cancers (*2, 3*). It is therefore conceivable that the HGF-HGFR/c-*met* system might have importance for development of cancers through autocrine or paracrine mechanisms. In fibroblast systems, i.e., NIH3T3 cells, however, every attempt so far to achieve transformation by transfecting the HGF gene has proved unsuccessful (personal communication and our own results). To ascertain whether this might be due to tissue or cell type specificity of action, we used a unique immortalized mouse hepatocyte cell line (MLE-10) established in our laboratory (*14*) to assess liver transforming potential.

Immortalized Mouse Hepatocyte Cell Line (MLE-10)

Since the cell line used is of key importance, some explanation about the process of establishment and characterization of the MLE-10 line is necessary.

For establishment of mouse hepatocyte cell lines, hepatocytes of carcinogen-untreated 8 week old male C3H mice were collected by portal perfusion and the Percoll centrifugation method, plated onto dishes and cultured with Waymouth medium supplemented with 10% fetal calf serum and the tumor promoter phenobarbital. After 6 months, colonies which developed were repeatedly subcloned leading to the establishment of 15 lines. Among these lines, 2 proved transplantable in nude mice with the remaining 13 not demonstrating any tumor development after 6 months of observation. None of these lines originally contained the activated c-H-*ras* with a point mutation at codon 61 which is very frequently detected in C3H mouse hepatomas. The non-transplantable 13 lines all became transplantable upon transfection of the activated c-H-*ras* gene. The tumors which developed in nude mouse subcutis were histopathologically diagnosed as hepatocellular carcinomas with mixed trabecular, glandular, sarcomatous, and undifferentiated patterns (*11, 15*). Clone number 10, MLE-10 was used for the present experiment. It has an overt hepatocytic nature including albumin and α-fetoprotein production in addition to histological features of the hepatocellular tumors described above.

Establishment of the HGF Producing Transfectants

Full-length rat HGF cDNA (the generous gift of Dr. Toshikazu Nakamura; *38*) was inserted into the vector pRc/CMV (purchased from Invitrogen, Inc.) and designated pRc/CMV-HGF (Fig. 1). pRc/CMV-HGF was transfected into MLE-10 by the calcium-phosphate method. Drug-resistant colonies were propagated and harvested colony by colony. After checking the integration and expression of exogenous HGF by Southern and Northern blotting, 5 established HGF producing lines were designated as MLE-10-HGF-1–5. pRc/CMV was similarly transfected and 3 established lines designated as MLE-10-CMV-1–3. Many characteristics of these and the original MLE-10, including growth capability in nude mice and on soft agar plates were examined.

Characteristics of HGF Transfectants

The results are summarized in Table I. The investigations of ELISA, scattering activity, and ^{125}I-HGF receptor binding were carried out in collaboration with Drs.

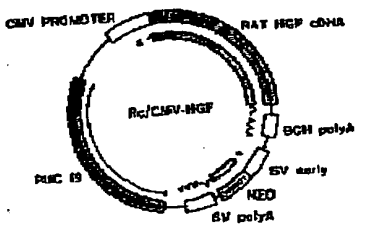

FIG. 1. Construction of the Rc/CMV-HGF plasmid
The plasmid introduced into MLE-10 (Rc/CMV-HGF) contained the following components: cytomegalovirus (CMV) promoter, full-length rat HGF cDNA, bovine growth hormone (BGH) intron and polyadenylation signal (poly-A), PUC19 replication origin, and β-lactamase gene. Full-length rat HGF cDNA was inserted into the vector Rc/CMV and expressed under the control of CMV promoter.

TABLE I. Characteristics of MLE-10 and Derived Lines

Cell line	HGF*	Soft agar growth	Tumorigenicity 2wks	Tumorigenicity 2mos	Scattering HGF−	Scattering HGF+	^{125}I-HGF receptor K_d	^{125}I-HGF receptor B_{max}	(HGFR)/met mRNA
1. MLE-10	<0.14	−	0/3	0/3	−	+	37	1180	+
2. CMV-1	<0.13	−	0/4	0/4	−	+	17	860	+
3. CMV-2	<0.08	−	0/3	0/3	−	+	37	1270	+
4. CMV-3	0	−	0/2	0/2	−	+	40	780	+
5. HGF-1	0.22	+	0/2	0/2	−	+	26	990	+
6. HGF-2	0.33	++	0/7	0/7	+	+	25	660	+
7. HGF-3	0.38	++	0/2	0/2	+	+	19	870	+
8. HGF-4	0.50	+++	3/3	3/3	+	+	ND	ND	+
9. HGF-5	0.69	+++	6/6	6/6	+	+	17	660	+

* HGF production: ng per 10^5 cells in 24 hr. ND: not determined.

TABLE II. Results of the Soft-Agar Assay

	No addition	Anti-HGF (10 μg ml^{-1})	Anti-HGF (80 μg ml^{-1}) + HGF (10 ng ml^{-1})	HGF (50 ng ml^{-1})
MLE-10	−	−	−	+
CMV-1	−	−	−	+
HGF-1	+	−	±	++
HGF-5	+++	−	++	+++

Hisao Tajima, Kunio Matsumoto, and Toshikazu Nakamura, of Osaka University, Japan.

All MLE-10-HGF lines produced HGF at levels ranging from 0.22 to 0.69 ng/10^5 cells at 24hr. Control lines of MLE-10 or MLE-10-CMVs did not produce HGF at all. All the HGF-transfected lines grew faster than control lines on dishes and acquired growth capability in soft agar. Colony size and numbers roughly paralleled HGF production levels. Colony formation was blocked by addition of anti-rat HGF antibody but reappeared after absorption of this antibody by recombinant rat HGF. Addition of HGF to the soft agar medium resulted in all control lines producing

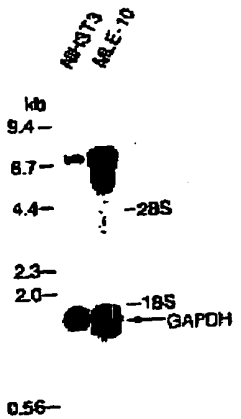

FIG. 2. Comparison of HGFR/*c-met* and GAPDH mRNA expression between MLE-10 and NIH3T3

The expression level of the HGFR/*c-met* gene in MLE-10 is about five or six times stronger than in the NIH3T3 line. In both cases 1 μg of poly-A RNA was electrophoresed.

colonies with the HGF-transfected lines making larger colonies (Table II). The highest producing lines, MLE-10-HGF-4 and 5, proved transplantable into the nude mouse subcutis. On the other hand, none of the other lines, including MLE-10-HGF-1, 2, or 3 produced tumors after 4 months of observation. The observed tumors demonstrated mixed trabecular, glandular, and sarcomatous features (Photo 1). When their histology was compared with those produced by activated c-H-*ras* gene-transformed cells, similarities were more impressive than differences. The growth rate of these tumors was also about the same as H-*ras*-transformed counterparts (at 2 months, 1.7 cm and 1.6 cm in diameter, respectively).

Scattering effect of HGF was obvious in this system. MLE-10-HGF-2–5 grew in a scattered fashion on the dishes. In contrast, the original MLE-10, MLE-10-CMV-1–3, and MLE-10-HGF-1 cells grew in sheets. After adding HGF exogenously to the medium, all the lines grew in a scattered fashion (Photo 2).

Examination of HGFR/*c-met* expression revealed high levels in the MLE-10 line, about 7 to 8 times higher than in NIH3T3 cells (Fig. 2). This was confirmed by ^{125}I-HGF binding assay. MLE-10 and all its derivatives expressed about the same amounts of high affinity HGF receptors as primary cultured rat hepatocytes. This high receptor expression and the responsiveness to exogenous HGF as clearly evidenced by the scattering effect suggest that the MLE-10 cells were transformable by an autocrine mechanism (*12*). This view is supported by the recent success of Rong *et al.* (*28*) in transforming NIH3T3 cells by co-transfecting both HGF and HGFR/*c-met* genes.

HGF and HGFR/c-met Expression in Cancer Cells

Next, we investigated the expression of HGF and HGFR/*c-met* in human cancers *in vivo* and *in vitro*. First, we examined cell lines of 8 gastric and 3 hepatic cancers, 2 melanomas, 1 breast and 1 thyroid cancer, and 6 other tumors by Northern blot analysis. HGFR/*c-met* gene expression was apparent in all lines although the expression levels varied. No HGF gene expression was found in any of the lines except very slightly in NIH3T3 cells. Thus co-expression of the HGF and HGFR/*c-met* genes was observed only in NIH3T3 cells.

Analysis of 10 mouse hepatomas then revealed both HGF and HGFR/*c-met* gene expression to be the same as or lower than in normal liver tissue. We further examined 27 human cancers including uterine cervix, uterine body, ovary, gastric carcinomas, and sarcomas resected in the Cancer Institute Hospital. None of them expressed detectable HGF mRNA. HGFR/*c-met* gene expression was also negative or only weak in all except for 5 gastric cancers. The observation of HGFR/*c-met* expression in gastric cancers was in agreement with the report of Tahara (*35*).

In the literature, however, HGF expression has been described in 3 out of 30 woodchuck hepatomas at higher than normal levels (*30*) and also in human glioblastoma, monocytic leukemia, lung cancer, and fibroblast cell lines (*27, 31, 41*). In contrast to the limited expression of HGF, the HGFR/*c-met* gene was found to be expressed rather frequently in various cancer lines, including examples derived from hepatomas, and especially highly in some gastric cancer lines. Recently, a lung cancer cell line coexpressing HGF and HGFR/*c-met* gene was also reported (*40*).

Growth Effect of HGF on Cell Lines

In the present investigation, we concentrated attention solely on the growth stimulating activity of HGF for MLE-10 cells. However, it is well known that the effect of HGF varies according to the cell. Thus, even among cells of hepatocytic nature, HGF acted positively on some cell lines including HuH-6 Clone 5 (*19*) but negatively on other cell lines including HepG2 (*19, 31, 36*). The mechanisms causing such conflicting effects in terms of growth are obscure. Since HGF has a morphogenetic or differentiating action, we are presumably looking at the balance between growth stimulation and growth supression in one and the same cell line.

It should be kept in mind that, as stressed by Tahara (*35*), growth factors other than HGF and cytokines including IL-1 and tumor necrosis factor (TNF) in the microenvironment may also stimulate HGF expression by mesenchymal cells and thus indirectly stimulate the growth of tumor cells expressing HGFR/*c-met*.

Conclusions

We demonstrated here the transforming activity of HGF by an autocrine mechanism utilizing MLE-10 cells. We also found relatively frequent expression of HGFR/*c-met* in various cancer cells. Although we failed to detect any cases showing co-expression of HGF and HGFR/*c-met*, such cases have been reported in the literature (*40*). It is therefore likely that the HGF-HGFR/*c-met* system, together with other growth factor and receptor systems, may in certain cases play an important role in carcinogenesis through autocrine and/or paracrine mechanisms.

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EXPLANATION OF PHOTOS

PHOTO 1. Histology of a MLE-10-HGF-5 cell-derived nude mouse tumor showing features of hepatocellular carcinoma with mixed trabecular, glandular, and sarcomatous patterns. (H&E, ×450)

PHOTO 2. Micrograph illustrating scattering effect of endogenous or exogenous HGF on lines. MLE-10, MLE-10-CMV-1–3, and MLE-10-HGF-1 cells grow with colony formation (a-1, MLE-10-HGF-1) and addition of exogenous HGF (2ng ml^{-1}) causes scattering (a-2). MLE-10-HGF-2–5 cells naturally grow in a scattered fashion (b-1, MLE-10-HGF-5) and exogenous HGF does not influence the pattern (b-2).

PHOTO 1

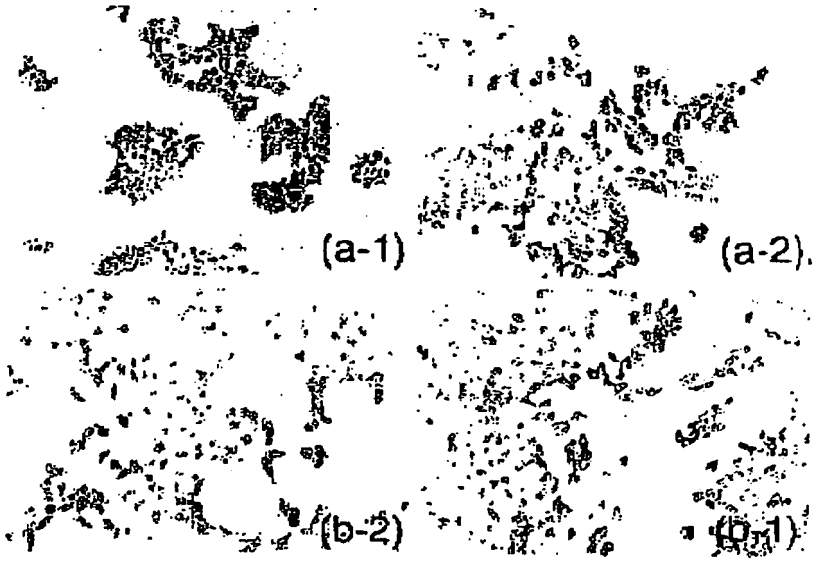

PHOTO 2

Mini-Review

The Many Faces of Hepatocyte Growth Factor: from Hepatopoiesis to Hematopoiesis

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RESEARCH performed over the past few years, in particular, has made it clear that, in addition to the liver, hepatocyte growth factor (HGF)[1] affects virtually every tissue in the body ranging from the nervous system to the immune and reticuloendothelial systems. Recent findings have also revealed that the biological responses of target cells to HGF are not confined to the induction of cell proliferation and motility per se but include a plethora of effects such as inhibition of cell growth, induction of morphogenesis, stimulation of T cell adhesion to endothelium and migration, enhancement of neuron survival, and regulation of erythroid differentiation. Furthermore, the discoveries of the HGF receptor as Met, of an HGF-related factor, called HGF-like protein, and of HGF-like's receptor as Ron, a transmembrane tyrosine kinase similar to the HGF receptor, have added yet more levels of complexity to the nature of HGF and its now growing family.

Structural Properties of HGF

Hepatocyte growth factor is a mesenchymally derived heparin-binding glycoprotein that is secreted as a single-chain (pro-HGF), biologically inert precursor. Under appropriate conditions such as tissue damage (21), pro-HGF is converted to its bioactive form by proteolytic digestion at a specific site within the molecule. This proteolytic digestion may be mediated by urokinase plasminogen activator (uPA) (27) or by a protease homologous to factor XII (21). Mature HGF is a heterodimer, consisting of a 60,000 M_r alpha and a 30,000 M_r beta chain held together by a single disulfide bond. The nucleotide sequences of human, rat, and mouse HGF cDNAs also predict that both chains of HGF are encoded by a single open reading frame resulting in a 728–amino acid polypeptide. The alpha chain of HGF contains a hairpin loop (of ~27 amino acids) at its amino terminus and four unique domains known as kringles, while its beta chain contains a serine protease-like structure (26). (For in depth review, see references 20 and 30.) The kringle motif, an 80–amino acid double-looped structure formed by three internal disulfide bridges, was first described for many of the enzymes involved in coagulation and fibrinolysis. Understandably, HGF resembles several coagulation/fibrinolytic related proteins such as plasminogen, but these proteins have no known growth potentiating activity comparable to HGF. Conversely, HGF has no known protease activity, since the characteristic amino acids normally present in the catalytic site of serine proteases have been mutated in HGF, while the consensus sequences which normally surround them still remain (26). These unique structural features of HGF have led to the assignment of HGF as the prototype of a new family of growth factors.

The HGF Receptor

Recent studies have shown that HGF transduces its multiple biological effects such as mitogenesis, motogenesis, metastogenesis and morphogenesis via activation of a transmembrane tyrosine kinase cell surface receptor known as Met (2, 40). The *met* protooncogene was cloned and sequenced before HGF itself had been cloned and was initially discovered as an activated oncogene based on its ability to transform normal fibroblast cell lines (4). The mature Met receptor is a heterodimer held together by disulfide bonds and consists of an alpha chain that is ~50,000 Da which remains entirely extracellular, and a large polypeptide chain with a molecular mass of 145,000 (named the beta chain) which traverses the plasma membrane and contains the intracellular tyrosine kinase domain. Both polypeptide chains of the Met receptor are derived posttranslationally from a single chain precursor by proteolytic cleavage at a specific site within the precursor molecule. (For review see reference 9). The HGF receptor (HGFR) is expressed in normal epithelium of almost every tissue; however, other cell types such as melanocytes, endothelial cells, microglial cells, neurons, hematopoietic cells, and a variety of tumor cell lines of various origins also express this receptor.

HGF, the Hepatocyte and Liver Regeneration

HGF's existence was originally postulated based on studies in which liver regeneration was surgically stimulated by removal of two-thirds of the liver in rats resulting in the appearance of a hepatocyte mitogen in the peripheral blood. Following two-thirds partial hepatectomy, plasma HGF levels are found to be 15- to 25-fold greater than

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1. *Abbreviations used in this paper*: HGF, hepatocyte growth factor; HGFR, hepatocyte growth factor receptor (Met); Ks, Kaposi's sarcoma.

those of the control animal. A similar rise in HGF also occurs sharply after acute liver injury after administration of chemicals such as carbon tetrachloride. The levels of HGF then decline within 24 h but remain elevated for the duration of the regenerative response. Analogous changes in plasma HGF levels have also been described after administration of chemicals to rats that augment liver DNA synthesis and liver weight (19), such as phenobarbital, dilantin, valium, and others that, in long term administration, function as liver tumor promoters, as well as in human liver disease whenever a massive loss of hepatocytes occurs such as in a condition known as acute fulminant hepatitis (37). These findings, as well as several other direct and indirect pieces of evidence, implicate HGF as an important paracrine and perhaps endocrine regulator of liver growth. (For review see reference 20).

The HGF/HGFR system may also influence other aspects of liver growth and development. In addition to its ability to stimulate proliferation and morphogenesis of mature hepatocytes, HGF is linked to hepatocyte stem cell proliferation and differentiation (8). As stem cells in the liver proliferate, nearby Ito cells also undergo cell division. These neighboring cells express HGF pointing to a possible paracrine effect of HGF on the growth of stem cells which express HGFR (8). Several studies also strongly suggest a role for HGF in liver embryogenesis. In situ hybridization studies reveal that HGF and HGFR expression is highest in the liver as compared to other tissues (Katyal and Michalopoulos, unpublished observations). Additionally, HGF protein is also present in the developing liver, especially in the hematopoietic cells (5), which may implicate HGF not only in liver formation but also in hematopoiesis (see below). Other supportive evidence includes the finding that HGF may be involved in the transformation of apolar embryonic hepatocytes into acinar structures prior to the appearance of the mature hepatocyte plate (34) which are the same morphological changes seen during liver regeneration and in hepatocytes cultured in type I collagen gels in the presence of HGF. Direct evidence on the role of HGF in liver growth and development comes from very recent investigations on transgenic mice homozygous for a null mutation in the HGF gene (knock-out mice). These studies have revealed that such animals do not survive beyond day 15 of embryonic development with most notable defects seen in liver formation and architecture primarily due to an extensive loss of hepatic parenchyma (31). Moreover, lack of HGF expression also affected the development of the placenta as was evidenced by a severe reduction in the number of trophoblasts (31, 38).

Other investigations have defined a fundamental role for HGF in mediating responses to tissue injury in other organs in adults. In animal models in which kidney (16) or lung (42) has been damaged experimentally, HGF expression is induced markedly. This increase in HGF mRNA is followed by increases in the level of pro-HGF and in its subsequent activation by proteolytic cleavage to the bioactive heterodimeric form in the damaged tissue (21). Additionally, when exogenous HGF is administered to these animals, it remarkably enhances the regeneration of the injured organ (20). These findings indicate that HGF is activated locally which may in part explain the lack of response to the biological effects of HGF in uninjured tissues. The discovery that the mRNAs for HGF (20) and the HGF receptor (23) are induced in stromal and epithelial cells, respectively, by inflammatory cytokines such as IL-1, IL-6, and TNFα also supports the idea that this ligand/receptor system is involved in mediating inflammatory responses to tissue injury. These studies have defined HGF as a major mediator of tissue repair and organ regeneration and underscores its potential use as a therapeutic agent for treating diseases such as acute liver or renal failure.

Biological Effects of HGF on Other Cell Types

The growth regulating effects of HGF on various cell types, other than hepatocytes, is now well-documented. (For review see references 20 and 30). One of the hallmark in vitro responses to HGF is the induction of cell motility and dissociation (scattering) of various normal and malignant epithelial cells. Based on this property, HGF was independently purified and characterized from the culture medium of fibroblast cell lines such as MRC-5, and was named scatter factor (35). Subsequent investigations, however, revealed that this molecule is identical to HGF (39). A third type of biological activity associated with HGF is its remarkable morphogenic effects on epithelial tissues. HGF is now believed to be most potent epithelial morphogen inducing formation of branching tubules and gland-like structures in epithelial cells derived from kidney or mammary tissue in vitro (24, 33).

Based on these properties, it has been postulated that HGF is a mediator of epithelial–mesenchymal interaction and interconversion. (For a review see reference 29). Most tissues either express HGF mRNA or contain HGF protein. Among these are blood (megakaryocytes, monocytes, leukocytes, and platelets), brain, bone marrow, liver, lung, kidney, placenta, spleen, and skin. The highest levels of HGF mRNA are detected in the adult lung, liver, skin, and spleen although the other tissues mentioned all contain detectable levels of HGF mRNA and/or protein. In general, HGF mRNA is expressed in stromal cells such as fibroblasts, smooth muscle cells, mast cells, macrophages, endothelial cells, leukocytes, and megakaryocytes of various tissues but not in epithelial cells. HGF receptor expression, on the other hand, is mainly detected in epithelial cells. This unique expression pattern in combination with the mitogenic, motogenic, and morphogenic properties of HGF support the idea that this ligand is an important paracrine mediator of the interaction between the epithelial and stromal compartments of various tissues during development and in the maintenance of homeostasis in adult tissues (29, 32).

HGF has also been shown to induce mesenchymal to epithelial conversion in fibroblasts overexpressing HGF and HGFR when these cells are injected into nude mice (36). In another investigation using cell lines derived from metanaphric ridge cells of mouse embryos, it was shown that HGF stimulates epithelial differentiation of these mesenchymal cells suggesting that this cytokine may be involved in the early commitment of cells in the kidney (15). The recent findings by Woolf et al. (41) show that simultaneous expression of HGF and HGFR occurs in the kidney mesenchyme during the early development of the mouse kidney and that anti-HGF antibody inhibits the differentiation of metanephric mesenchymal cells into the epithelial

precursors and subsequent nephrogenesis when added to metanephric organ cultures. Other studies have also implicated HGF in early embryological processes such as the formation of the primitive streak and induction of neural tissues as demonstrated by studying HGF and HGFR expression in Xenopus and chick during early stages of development and by ectopic application of HGF in a chick embryo model. (For review see reference 20.)

The potential participation of HGF in organogenesis and the later stages of embryonic development has also been well documented by studying HGF and HGFR expression in rodents (5, 32). As stated, recent studies have shown that the disruption of the HGF gene results in embryonic lethality, primarily due to defects in proper development of the placenta and liver (31, 38). In these studies, however, other organs and tissues where HGF and HGFR are reportedly expressed during embryogenesis (such as lung, kidney, and the central nervous system), appeared normal at the time of death (day 15 of gestation). This may indicate that either HGF is not essential for the early stages of embryogenesis or that compensation or redundancy exists in the HGF signaling network. It should be noted that further analysis of these animals has been hampered due to their death in utero preventing additional investigations on the functions of HGF in other processes such as terminal differentiation and maturation or regeneration of other tissues (31).

It should be emphasized that although epithelial cells are one of the major targets of HGF, as more investigations are conducted, it is becoming clear that nonepithelial cell types such as hematopoietic, lymphoid, neural, and skeletal muscle cells also respond to the multifaceted actions of HGF. The first clue indicating that HGF may be involved in hematopoiesis came from studies on progenitor-enriched murine bone marrow cells and on several murine myeloid progenitor tumor cell lines blocked in the early stages of myeloid differentiation. Such investigations revealed, first, that these cells express the HGFR, and second, that HGF synergizes with IL-3 or GM-CSF to support the growth of these cells in culture (17, 22). Conflicting results, however, were obtained by these two investigations with regard to whether HGF alone stimulates mitogenesis in myeloid progenitor cell lines. Although HGF synergized with other factors to stimulate growth of progenitor cells, it apparently did not influence the pattern of myeloid differentiation since the ratio of macrophages to granulocytes in resultant colonies remained similar to those obtained with IL-3 or GM-CSF alone (17).

Galimi et al. (10) recently reported that the HGF receptor is present in a small fraction of highly-enriched hematopoietic progenitor cells from human bone marrow and peripheral blood and showed that, in the presence of erythropoietin, HGF induces the formation of colonies along the erythroid lineage when cultured in vitro. However, in the presence of erythropoietin and stem cell factor, it was demonstrated that HGF supports the growth of multipotent colonies (granulocyte-erythroid-megakaryocyte) rather than recruiting erythroid precursors.

The differences in the results of the experiments described above may be due to variations in technique such as culture conditions (i.e., presence or absence of particular cytokines and doses), purity of cells, or simply because so few studies have addressed HGF's involvement in hematopoiesis. Additionally, whether HGF's mitogenic/differentiation effects on hematopoietic stem cells are mediated through the HGFR directly or whether HGF through its receptor causes secretion of other modulating cytokines has not been addressed in studies thus far completed. Regardless of how HGF elicits such responses, the fact that hematopoiesis is altered at all in the presence of HGF deserves further study.

The role of the HGF family in hematopoiesis and reticuloendothelial cell function is further underscored by other findings. It has been shown that the HGF-like receptor (Ron) is highly expressed in hematopoietic stem cells (12) and monocytes (11). Moreover, HGF-like protein (also known as macrophage stimulating protein) was demonstrated to elicit monocyte migration through Ron activation (11). Biological activities affecting reticuloendothelial cells have also been described for HGF and the HGFR using in vitro models. These include activation of the oxidative response of human neutrophils (13), promotion of adhesion and migration of a subset of human T cells (1), and enhancement of humoral immune responses in murine B cells (6).

Recently, in vivo studies in which HGF was implanted into rabbits have demonstrated that HGF has angiogenic activities (3). Interestingly, HGF has also been linked to Kaposi's sarcoma (KS), a form of human neoplasm in which the cellular origin has not been defined, but is believed to be an endothelial derivative. Several lines of evidence support this possibility. First, HGF is secreted by HTLV-II-infected T lymphocytes; secondly, HGF induces endothelial cells to convert to a Kaposi sarcoma tumor cell-like phenotype; thirdly, antibody against HGF inhibits the growth of KS cells in culture; and lastly, HGF is present in KS lesions (25).

The presence of HGF and its receptor in specific regions of the developing and adult mammalian nervous system points to the fact that this ligand/receptor system may have a neurotrophic function (14). Recently, HGF was shown to promote the survival of motor neurons and enhance the neurotrophic function of ciliary neurotrophic factor. In other studies, Krasnoselsky and colleagues reported that rat sciatic nerve Schwann cells express the HGF receptor and strongly respond to the mitogenic effects of HGF in culture (18).

HGF has also been examined in the context of endocrine function. Studies on the effects of HGF on the endocrine system show that HGF may contribute to the formation and maintenance of organs involved in hormone secretion such as the pancreas and the thyroid. Using primary organ cultures of human fetal pancreas, Otonkoski et al. have recently reported that HGF is the most potent inducer of β-cell proliferation and formation of islet-like cell clusters subsequently resulting in insulin production in vitro (28). HGF also seems to regulate the growth and function of the thyroid gland as revealed by in vitro studies (7).

Although the early work on HGF was confined almost exclusively to one organ, the liver, researchers with wide-ranging scientific interests have helped over the years to define the multifaceted functions and target cells of HGF. Clearly, however, much remains to be learned about HGF, and more biological and physiological roles for this growth factor will undoubtedly be revealed.

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Emerging Multip tent Aspects of Hepatocyte Growth Factor

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Specific tissue interactions between epithelia and mesenchyme (or stroma), e.g., epithelial-mesenchymal (or -stromal) interactions mediate crucial aspects of normal development and tissue regeneration. These events affect tissue induction, organogenesis, cell movement, and morphogenesis of multicellular structures. Extensive and diverse studies have established that hepatocyte growth factor (HGF), a ligand for the c-*met* protooncogene product of receptor tyrosine kinase, is a mesenchymal- or stromal-derived multipotent polypeptide which mediates epithelial-mesenchymal interactions. During embryogenesis, HGF supports organogenesis and morphogenesis of various tissues and organs, including the liver, kidney, lung, gut, mammary gland, tooth, skeletal system, *etc.* In adult tissues, HGF elicits a potent organotrophic function which supports regeneration of organs including the liver, kidney, and lung. In the brain, HGF is a new member of the family of neurotrophic factors. In neoplastic tissue, HGF is involved in tumor invasion and metastasis, through tumor-stromal interactions. While HGF was originally identified as a potent mitogen for mature hepatocytes, the biological functions of this factor reach far beyond the original identifications. Such being the case, use of HGF for purposes of therapeutics is being given increasing attention.

Key words: c-*met*, epithelial-mesenchymal interactions, HGF, morphogenesis, organogenesis, organ regeneration.

Growth factors which share multipotent characteristics regulating proliferation, motility, and differentiation of cells are members of critical molecules responsible for complex biological processes, including embryogenesis, angiogenesis, tissue regeneration, and malignant transformation. Hepatocyte growth factor (HGF) was initially identified in a partially purified form to be a potent mitogen for mature hepatocytes in primary culture (*1-4*). HGF was thereafter completely purified (*5-7*) and was molecularly cloned in 1989 (*8, 9*).

In 1990 to 1991, independent approaches led to isolation of bioactive molecules using different assay method were unexpectedly joined upon molecular cloning of factors. The cloning of cDNAs for scatter factor (*10*), tumor cytotoxic factor (*11*), and fibroblast-derived epithelial growth factor (*12*) revealed these molecules to be identical with HGF. Scatter factor was originally identified as a fibroblast-derived factor which "scatters" tightly growing epithelial cell colonies (*13*). Tumor cytotoxic factor proved to be fibroblast-derived factor which inhibits growth of certain species of carcinoma cells (*14*). In 1991, a fibroblast-derived epithelial morphogen which induces branching tubulogenesis in epithelial cells also proved to be HGF (*15*), and a natural ligand for the receptor-tyrosine kinase, c-*met* protooncogene product was identified as HGF in the same year (*16, 17*).

While HGF is a potent hepatotrophic factor responsible for vigorous regeneration of the liver, it has become a well characterized multipotent growth factor which targets a wide variety of cells (reviewed in Refs. *18-22*). HGF has "trophic" roles for regeneration and maintenance of various tissues and organs (*18, 23*). Recent extensive studies on expression and functional analysis of HGF during embryogenesis revealed a distinct aspect of this factor as a mediator in morphogenic epithelial-mesenchymal interactions essential for organogenesis (*24-34*). Based on its potent motogenic (enhancement of cell motility) and angiogenic activities, it seems clear that HGF is involved in growth, invasion, and metastasis of tumor cells. In this review, we will focus on unique multipotent aspects of HGF as a mediator in specific cell-cell interactions.

Biochemical characteristics of HGF and c-Met

HGF is a heterodimer with a 69 kDa α-chain and a 34 kDa β-chain, linked by a single disulfide bridge (*5-7*) (Fig. 1). The α-chain contains the N-terminal hairpin structure and four homologous "kringle domains" and the β-chain has serine protease-like motif. Thus, HGF has a structural homology with plasminogen (*8, 12, 35-37*). But HGF has no serine protease activity, while plasminogen and its active form, plasmin share no biological activities of HGF (*6*). HGF is translated from a single mRNA, as a single chain preproHGF. Extracellular processing by specific serine protease, HGF-activator or HGF-converting enzyme (*38, 39*), results in conversion from a biologically inactive form to active two-chain mature HGF.

There are two known distinct forms of naturally occurring variant HGF, biosynthesized through alternative splicing of pre-mRNA; one form is deleted with 5 amino acids in the first kringle domain (*12, 37*) while the other

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Abbreviations: HGF, hepatocyte growth factor; HLP/MSP, HGF-like protein/macrophage stimulating protein.

form consists of only the N-terminal hairpin domain and two kringle domains (*40, 41*). The former has mitogenic and motogenic activities whereas the latter form has no mitogenic activity but does have motogenic activity (*42, 43*). This smaller variant is likely to be a minimum unit for binding to the c-Met/HGF receptor, with a relatively high-affinity. In accordance with this, deletion of the N-terminal hairpin domain, the first kringle domain, or the second kringle domain in the HGF molecule results in a total loss of biological activities (*43, 44*).

The tumorigenic met oncogene was initially isolated from chemically transformed human osteosarcoma cells. Although the primary structure of the c-*met* protooncogene product predicted it to be a receptor-type tyrosine kinase, it remained orphan (or lonesome) receptor until two research groups independently identified its natural ligand to be HGF (*16, 17*). The c-Met/HGF receptor is heterodimeric molecule composed of a 50 kDa α-chain and a membrane spanning 145 kDa β-chain which contains the intracellular tyrosine kinase domain (*45*). The Met/HGF receptor, when autophosphorylated in response to HGF, binds a number of substrate containing the Src homology region 2 (SH2) domains such as phosphatidylinositol 3-kinase, Grb-2(Ash)/Sos complex, Ras GTPase activating protein, pp60^{src}, and phospholipase C-γ (*46, 47*). These intracellular signaling molecules associate with a docking site of the tandemly arranged C-terminal tyrosine residues 1349 and 1354. Mutation of these tyrosine residues results in loss-of-function mutation (*46, 48, 49*), while mutation of the juxtamembrane tyrosine residue suppresses the loss-of-function mutation of the Met/HGF receptor (*49*).

While growth factors are often classified into certain families, based on structural similarity, cDNA encoding an unique protein with a similar domain structure to HGF was isolated and the putative protein was termed HGF-like protein (HLP) (*50*). HLP was later shown to be an molecule identical with macrophage stimulating protein (MSP) (*51, 52*), originally purified from human serum. On the other hand, the c-Met/HGF receptor has two distinct family members; Ron and Sea (*53, 54*). Ron tyrosine kinase was identified as a specific receptor for HLP/MSP (*55-57*), but a ligand for Sea tyrosine kinase remains to be identified (Fig. 2).

Biological activities

The growth-regulating activity of HGF for various cell types has been well-characterized, as described in Table I. HGF has mitogenic activity for epithelial cells (*12, 18, 58-68*), endothelial cells (*69-71*), some stromal cells (*72-76*), and various species of carcinoma cells (*58, 78-82*). HGF

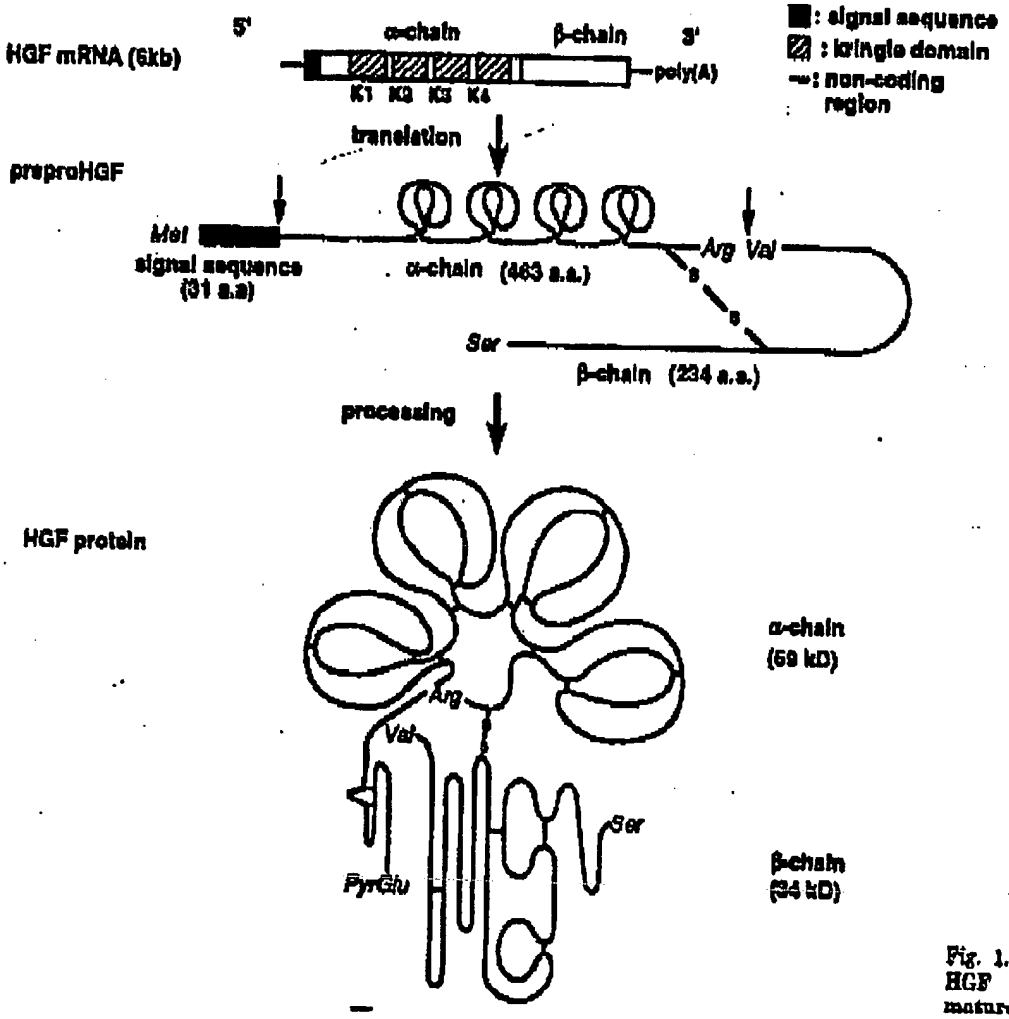

Fig. 1. Schematic structure of HGF mRNA, preproHGF, and mature HGF.

also has an angiogenic activity when implanted *in vivo* (*70, 71*). Recent studies revealed that HGF is also involved in hematopoiesis (*72, 74, 76*), chondrogenesis, and bone remodeling (*75, 77*). HGF stimulates proliferation of hematopoietic progenitor cells and enhances the formation of colonies toward erythroid lineage or granulocyte-erythroid-megakaryocyte lineage (*72–74, 76*). HGF enhances growth and differentiation of osteoclastic cells at the terminal stage (*77*). Articular chondrocytes are target cells of HGF and HGF mRNA is expressed at presumptive articular regions during development (*75*). *In vitro* and *in vivo* studies provided that HGF has anti-tumor activity for certain species of carcinoma cells, and in particular, growth of most hepatoma cells is inhibited by HGF (*14, 83–85*).

Cell movement is an important process during embryogenesis, wound healing, and tumor invasion. Although some growth factors are known to enhance cell motility, HGF is one of most potent motogens to induce dissociation and cell movement in various types of cells (Table I) (*13, 30, 59, 70, 78, 79, 81, 86–89*). The motogenic activity of HGF is mediated by activation of small GTP-binding proteins, Rho, Ras, and Rac (*90–93*). Disruption and regulation of cell-cell and cell-matrix interactions are related to the phosphorylation of E-cadherin-associated molecules (*β*-catenin, plakoglobin, and p120) (*94, 95*) and focal adhesion kinase ($p125^{FAK}$) (*88*), respectively. HGF also disrupts intercellular communications mediated by gap junctions (*96, 97*).

Among the multipotent characteristics of HGF, the morphogenic activity is notable and unique. This activity was initially noted in three-dimensional collagen gel cultures using MDCK cells derived from renal epithelium, wherein HGF induces branching tubular structures (*15*). Induction of similar branching tubules and gland-like structures in epithelial cells also occurs in other cells, including cell lines derived from hepatic duct and mammary gland (*29, 30, 80*). Therefore, HGF is an important factor regulating morphogenic processes during development and tissue reconstruction (see below).

Several ligands for receptor-tyrosine kinases have distinct neurotrophic actions in the brain, including members of the nerve growth factor family, basic fibroblast growth factor, and epidermal growth factor. HGF and c-Met/HGF receptor are expressed in various regions of the brain (*98, 99*). HGF activates Ras in neurons (*100*) and acts as a potent survival factor for primary cultured neurons and PC12 pheochromocytoma cells (*99, 101, 102*), all findings to support the thesis that HGF belongs to the family of neurotrophic factors. Likewise, HGF acts as a mitogen for Schwann cells (*103*).

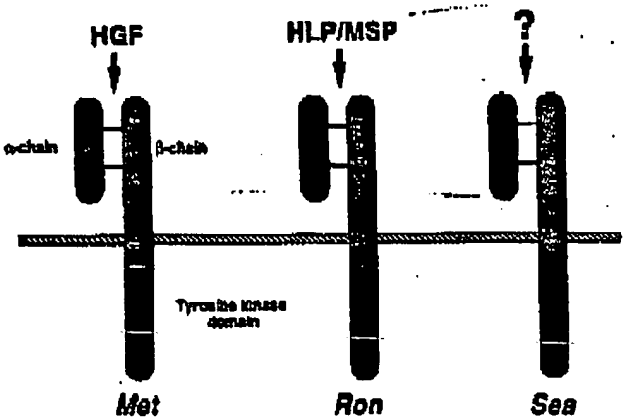

Fig. 2. Ligand-receptor relationship in molecules of HGF and c-Met/HGF receptor families.

HGF in epithelial-mesenchymal interactions and development

Interactions between epithelium and mesenchyme, *e.g.*, epithelial-mesenchymal interactions mediate crucial aspects of normal development, affecting tissue induction, organogenesis, and morphogenesis of specific multicellular structures. Development and morphogenesis of various organs and tissues, including kidney, lung, liver, pancreas, limb, tooth, mammary gland, hair follicle, *etc.* depend on epithelial-mesenchymal interactions. A conceptual frame-

TABLE I. Typical biological activities of HGF and target cells.

Biological activity	Target cells
Mitogenic	
	Hepatocytes
	Hepatoblast-like cells
	Hepatic ductular epithelial cells
	Renal tubular cells
	Keratinocytes
	Hair cells
	Melanocytes
	Gastric epithelial cells
	Corneal epithelial cells
	Bronchial epithelial cells
	Alveolar type II epithelial cells
	Thyroid cells
	Mammary gland epithelial cells
	Schwann cells
	Pancreatic β cells
	Placental cytotrophoblasts
	Prostate epithelial cells
	Osteoclast-like cells
	Vascular endothelial cells
	Articular chondrocytes
	Hematopoietic progenitor cells
	Gallbladder cancer cells, *etc.*
Motogenic	
	Renal epithelial cells
	Hepatic ductular epithelial cells
	Keratinocytes
	Thyroid cell
	Mammary gland epithelial cells
	Vascular endothelial cells
	Articular chondrocytes
	Myogenic precursor cells
	Oral squamous carcinoma cells
	Gallbladder carcinoma cells
	A431 epidermoid carcinoma, *etc.*
Morphogenic	
	Renal epithelial cells
	Hepatic epithelial cells
	Mammary gland epithelial cells
	Colon carcinoma cells, *etc.*
Promotion of cell survival	
	Neurons
	PC12 rat pheochromocytoma cells
Tumor inhibition	
	Hepatoma cells (HepG2, *etc.*)
	B6/F1 melanoma cells
	KB squamous carcinoma cells, *etc.*

work of epithelial-mesenchymal interactions was established in the 1950s and 1960s, but molecular mechanisms responsible for these interactions have not been elucidated. Recent extensive works on HGF have established that HGF is a mesenchymal-derived mediator in epithelial-mesenchymal interactions.

During kidney development, the first interaction between epithelial ureteric bud and mesenchymal metanephric blastema is essential for the development of the kidney. Epithelial cells derived from ureteric bud form branching collecting tubules and mesenchymal cells at the tip of collecting tubules convert to epithelial cells that form the nephron. During organogenesis of the kidney, c-Met/HGF receptor mRNA is expressed in epithelial cells, while HGF mRNA is expressed in mesenchymal cells in close proximity to renal epithelial cells (*24, 26*). A specific antibody against HGF inhibits both morphogenesis of the kidney in organ culture system and differentiation of metanephric mesenchymal cells into epithelial precursors of the nephron (*25, 26*). Together with *in vitro* induction of branching tubulogenesis by HGF, this factor is a mesenchymal-derived morphogen for renal epithelial cells and is involved in transdifferentiation from mesenchymal to epithelial cells. Likewise, HGF and c-Met/HGF receptor mRNA are expressed in mammary gland tissue, and HGF potently promotes the formation of branching duct-like structures by mammary gland epithelial cells *in vitro* (*29-31*). Therefore, HGF may mediate inducing effects of mesenchyme (or stroma) on mammary gland development.

The potential participation of HGF in organogenesis was also demonstrated by disruption of the HGF gene (*27, 28*). In the homozygous mutant mice of HGF gene, embryos are lethal, due to defected development of the placenta (*28*) or both placenta and liver (*27*). Likewise, in c-*met* homozygous mutant mouse embryos, development of the liver and placenta was defected (*89*). These defects are in consistent with the finding that HGF is a potent mitogen for placental cytotrophoblasts (*64*), as well as hepatocytes. The essential role of HGF in liver development has recently been demonstrated using *in vivo* loss-of-function mutation in the *Xenopus* embryo. Overexpression of mutant c-Met/HGF receptor of tyrosine kinase-minus (TK$^-$-Met) in *Xenopus* embryos resulted in liver defects and impaired development of pronephros, gut, and skeletal morphogenesis in tail regions (Aoki *et al.*, submitted). These results indicate that HGF and the c-Met/HGF receptor are highly conserved molecules, at least with regard to development of the liver, from amphibians to mammals. In contrast, in transgenic mice that express HGF specifically in the liver, a new population of small hepatocytes (presumably blastic hepatocytes) appears in the liver (*104*), thereby indicating that HGF may be involved in proliferation of hepatoblast-like cells (*61, 105*).

Localization of HGF and c-Met/HGF receptor mRNA in various tissues indicates that functional coupling between HGF and the c-Met/HGF receptor is important for development, morphogenesis, and migration of cells in other tissues, including limb, branchial arches, lung, tooth, and bone. Figure 3 shows the *in situ* localization of HGF and c-Met/HGF receptor mRNA in developing lung of day 13 of the rat embryo. The c-Met/HGF receptor mRNA is specifically localized in bronchial epithelial cells (Fig. 3, C and D), while HGF mRNA locates in surrounding mesenchymal cells (Fig. 3, A and B). Antisense HGF oligonucleotide specifically inhibits branching tubulogenesis of the developing lung *in vitro* (our unpublished data). Thus, HGF is a mesenchymal-derived factor for branching morphogenesis during lung development. Similarly, the functional coupling between HGF and c-Met/HGF receptor supports tooth development (*32*). The c-Met/HGF receptor is expressed in epithelial tissue while HGF is expressed in mesenchymal tissue in tooth germ, and antisense HGF oligonucleotide specifically induces abnormal tooth morphogenesis in organ culture system (*32*). Expression pattern of HGF and c-*met*, and biological activities of HGF implicate HGF in skeletal morphogenesis and chondrogenesis. HGF is mitogenic and motogenic for chondrocytes (*75*), and HGF and c-*met* are expressed in chondrogenic regions, including rib, limb joints, and branchial arches (*33, 34*). In met$^{-/-}$ embryos, migration of myogenic precursor cells into the limb bud, diaphragm, and tip of tongue is impaired, and as a consequence, skeletal muscles of the limb and diaphragm do not form (*89*). These observations mean that HGF is involved in migration of cells during development. In the chick embryo, HGF is involved in early steps of neural induction, presumably by inducing or maintaining

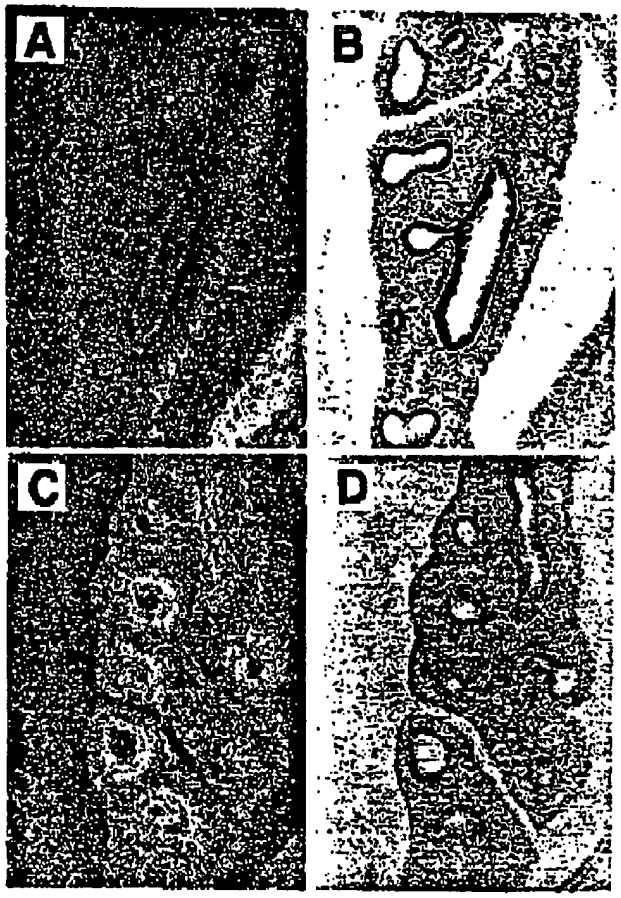

Fig. 3. Expression of HGF and c-Met/HGF receptor mRNA in developing rat lung. Localization of HGF mRNA (A, B) and c-Met/HGF receptor mRNA (C, D) was analyzed by *in situ* hybridization using day 13 rat embryo. B and D indicate bright field views for A and C, respectively.

competence of the epiblast to respond to neural-inducing signals (*106–109*).

Organotrophic roles

Regeneration of the liver is one of the most dramatic phenomena in higher animals. When 70% of the liver is resected, the cells in the remaining liver rapidly proliferate and the original liver mass and functions are restored within a week. As partial purification of HGF was originally done using peripheral blood of partially hepatectomized rats, HGF was considered to be a humoral hepatotrophic factor which enhances liver regeneration. During the last 10 years, the hepatotrophic roles of HGF have been well-established. HGF is now seen to have the role of organotrophic factor for regeneration of other tissues and organs (*19–23*).

Liver injuries can be induced in rats or mice by means of partial hepatectomy, ischemia, liver crush, or administration of hepatotoxins such as CCl_4 and α-naphthyl-isothiocyanate. Expression of HGF mRNA rapidly increases following the onset of these injuries in the injured liver and distant intact organs such as the lung and spleen. The liver is composed of several types of cells, including parenchymal hepatocytes, sinusoidal endothelial cells, Kupffer cells (liver macrophages), Ito (fat-storing) cells, and bile duct epithelial cells. Cell fractionation and *in situ* hybridization revealed that HGF is expressed in non-parenchymal stromal cells such as Kupffer cells, sinusoidal endothelial cells, and Ito cells, but not in parenchymal hepatocytes, indicating that this factor acts through a paracrine mechanism. Additionally, up-regulation of HGF mRNA in intact organs, together with a marked increase in blood HGF levels, means that an endocrine-related mechanism is likely to be functioning in liver regeneration. Elevated levels of plasma HGF were also well-demonstrated in patients with hepatic diseases (*110, 111*).

Based on a wide spectrum in target cell specificity of HGF, the involvements of HGF in regeneration of other organs was noted. Expression of HGF is rapidly induced after injuries in the kidney and lung (*112, 113*). In analogy with the case of liver injury, non-epithelial stromal cells produce HGF. Taken together with *in vitro* and *in vivo* mitogenic actions of HGF for renal (*114, 115*) and lung epithelial cells (*60, 116*), HGF seems to trigger regeneration of these organs at least through a paracrine mechanism. Therefore HGF is a stromal-derived mediator responsible for organ regeneration. Changes in blood HGF levels were noted in patients with renal diseases (*117*) and in patients treated by renal transplantation (*118*).

Expression of HGF is regulated by various factors. Interleukin-1, platelet-derived growth factor, acidic and basic fibroblast growth factor, epidermal growth factor, prostaglandins, and heparin are potent inducers of HGF expression (*119–121*). In contrast, transforming growth factor-β1 and glucocorticoids suppress the gene expression of HGF (*122, 123*). Although these regulatory molecules are likely to have distinct roles, the regulatory network for expression of HGF may be involved not only in organ regeneration but also in epithelial-mesenchymal and tumor-stromal interactions during organogenesis and tumor progression (see below), respectively.

Direct evidence for the organotrophic roles of HGF has been obtained from *in vivo* studies and these studies suggested potential therapeutic strategies using recombinant HGF. Administration of HGF to experimental animals with liver injury strongly enhanced liver regeneration (*124–126*), and importantly, HGF suppressed the onset of hepatic dysfunction (*124, 127*). Likewise, HGF enhances renal regeneration and suppresses the onset of acute renal failure caused by renal toxins, renal ischemia, or unilateral nephrectomy (*114, 115*). More importantly, HGF prevented the onset of liver fibrosis/cirrhosis and abrogated lethal hepatic dysfunction due to chronic liver injury (*128*). Mitogenic, motogenic, and morphogenic activities, all of which are required for reconstruction of tissue architecture, no doubt are responsible for the organotrophic functions of HGF.

HGF in tumor–stromal interactions

Because of its profound effects on cell growth, motility, and angiogenesis, HGF is implicated in the growth, invasion, and metastasis of tumor cells. As establishment of an autocrine loop of growth factors and their receptors is involved in tumorigenic transformation of cells, gene transfer experiments indicate that autonomous activation of c-Met/HGF receptor results in tumorigenic transformation (*129–132*). Stable transfection of the HGF gene in *met*-expressing epithelial cells (*130–132*), and the c-*met* gene in HGF-producing fibroblasts (*129*) both confer *in vivo* tumorigenicity in these cells. Such an autocrine activation of the Met/HGF receptor is found in certain tumor cells derived from cancer patients (*87, 133*), however, most carcinoma cells derived from epithelial tissues express c-Met/HGF receptor but do not express the HGF gene. This may mean that autocrine activation of the Met/HGF receptor is restricted to certain species of tumor cells.

Studies indicate the particular importance of stromal-derived HGF in invasion and metastasis of carcinoma cells. Growth and invasive potentials of tumor cells are influenced by their interactions with normal stromal fibroblasts (*134–136*). *In vitro* invasion of carcinoma cells into the collagen gel matrix was induced in co-cultivation with stromal fibroblasts (*135*), and fibroblasts can produce migration-stimulating factor (*137*). Although molecular mechanisms underlying these tumor-stromal interactions are of current interest to tumor biologists, one fibroblast-derived invasion factor is known to be HGF (*88*) and HGF induces invasion of various types of carcinoma cells *in vitro* (*78, 79, 81, 86, 87*). In addition to stromal fibroblast-derived HGF, we recently found that carcinoma cells secrete inducing factors for HGF expression in fibroblasts (Matsumoto *et al.*, submitted) and the presence of such inducing factor(s) was also noted by other workers (*138, 139*). Therefore, HGF seems to be a predominant stromal-derived invasion factor for carcinoma cells. The mutual interaction between HGF-expressing stromal cells and Met-expressing carcinoma cells mediated by HGF and its inducers may result in an acquisition of invasive phenotype in tumor cells. The epithelial-mesenchymal (or -stromal) interactions mediated by HGF are likely to be functional in tumor-stromal interactions, as well as in tissue regeneration.

Perspective and future directions

The biological and physiological functions of HGF have been much greater than expected (Fig. 4). However, much

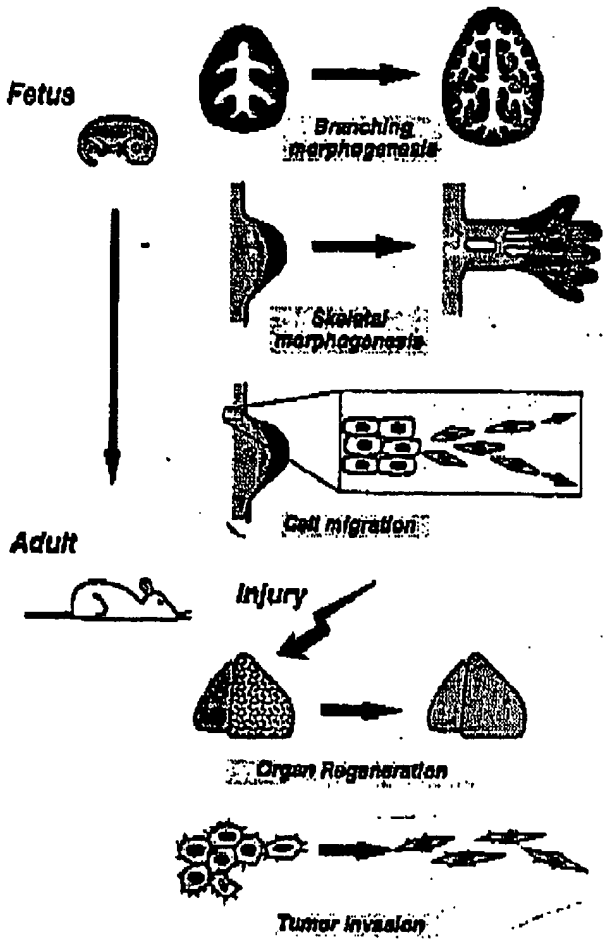

Fig. 4. Pleiotropic roles of HGF during embryogenesis, organ regeneration, and tumor progression.

work remains to be determined how HGF exerts its highly diversified activities and how HGF is involved in constructing an organized multicellular tissue structures (*e.g.*, branching tubules).

Target cells of HGF are distributed widely and specific biological roles of HGF for the development and homeostasis of each tissue need to be studied. Although localization and *in vitro* analysis indicate that HGF may well play a neurotrophic role in the brain, specific roles of HGF for the maintenance and regeneration of the central nervous system, and also for the inductive processes, development, and network formation of neural cells are of general interest.

Because of its organotrophic functions, HGF may well have therapeutic potential for disorders of the liver and kidney. HGF is highly effective for chronic and often incurable hepatic disease, *e.g.*, liver fibrosis/cirrhosis. Application of the HGF gene as a therapeutic for chronic diseases may be feasible. The generation and application of antagonistic molecules of HGF may prove to be therapeutic in inhibiting tumor invasion and metastasis.

The biological functions of HLP/MSP and Ron are still uncharacterized. Identification of novel members in HGF and the Met family, and elucidation of biological functions of HLP/MSP will shed light on the biological significance of HGF family molecules in embryogenesis, organogenesis, and tissue regeneration.

Due to space limitation, the papers of some scientists may not have been cited. Nevertheless, we are entirely grateful to all our colleagues for "working on HGF." Gratitude is extended to M. Ohara for helping us to write up this review.

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